

Cyclin-dependent kinase inhibitors

Yun Dai and Steven Grant

Cell-cycle dysregulation is one of the cardinal characteristics of neoplastic cells. For this reason, small molecule inhibitors targeting cyclin-dependent kinases (CDKs), of which flavopiridol is a prototype, have been the focus of extensive interest in cancer therapy. In addition to inhibiting cell-cycle progression, these agents exhibit a variety of other activities, including the induction of cell death. Recently, several novel mechanisms of action have been ascribed to the CDK inhibitor flavopiridol, including interference with transcription, most likely through disruption of P-TEFb (i.e. the CDK9/cyclin T complex), and induction of apoptosis, possibly a consequence of downregulation of various anti-apoptotic proteins. It has also been observed that combining CDK inhibitors with either conventional cytotoxic drugs or novel signal transduction modulators dramatically promotes neoplastic cell death in a variety of preclinical models. Efforts are underway to uncover inhibitors that selectively target specific CDKs and to develop these as a new generation of antitumour drugs. For all of these reasons, it is likely that interest in CDK inhibitors as antineoplastic agents will continue for the foreseeable future.

Addresses

Division of Hematology/Oncology, Medical College of Virginia,
Virginia Commonwealth University, MCV Station Box 230,
Richmond, VA 23298, USA
Correspondence: Steven Grant; e-mail: stgrant@hsc.vcu.edu

Current Opinion in Pharmacology 2003, 3:362–370

This review comes from a themed issue on
Cancer

Edited by Philip Frost and Barton A Kamen

1471-4892/\$ – see front matter

© 2003 Elsevier Science Ltd. All rights reserved.

DOI 10.1016/S1471-4892(03)00079-1

Abbreviations

CDK	cyclin-dependent kinase
CDKI	cyclin-dependent kinase inhibitor
IAP	inhibitor of apoptosis
P-TEF	positive transcription elongation factor
RNAPII	RNA polymerase II
VEGF	vascular endothelial growth factor

Introduction: rationale for the development of CDK inhibitors as antineoplastic agents

Cyclin-dependent kinases (CDKs) represent key molecules involved in regulation of the cell-cycle. CDKs are serine/threonine kinases that become active only when associated with a regulatory partner (e.g. cyclins or other proteins). CDK/cyclin holoenzymes are activated by phosphorylation, which is catalyzed by CDK-activator

kinase (CAK). The activity of CDKs is negatively regulated by direct interactions with proteins referred to as CDK inhibitors (CDKIs). CDKIs are divided into two major families: the INK4 (inhibitor of CDK4) family, including p16^{ink4a}, p15^{ink4b}, p18^{ink4c} and p19^{ink4d}, which specifically inhibit cyclin D-associated kinases (CDKs 4 and 6); and the Cip/Kip (kinase inhibitor protein) family, consisting of p21^{cip1/waf1}, p27^{Kip1} and p57^{Kip2}, which inhibit most CDKs. Dysregulation of molecules controlling the cell-cycle plays an important role in tumour pathogenesis. For example, alterations in the cascade involving CDK4, CDK6, cyclin D, INK4, pRb and E2F have been observed in more than 80% of human cancers. Genetic abnormalities of other CDKs (e.g. 1, 2 and 7), cyclins (e.g. A and E) and CDKIs (e.g. p27^{Kip1} and p21^{waf1}) have also been reported in many human cancers. Moreover, enforced perturbations in CDKs and/or CDKIs can promote neoplastic transformation and/or enhance the carcinogenic properties of oncoproteins. Thus, CDKs represent very attractive targets for cancer therapy. More than 50 pharmacological CDK inhibitors have been described, some of which have potent antitumour activity. Furthermore, multiple CDK inhibitors are currently undergoing preclinical and clinical evaluation, and the search for new CDK inhibitors continues. Recent advances in the development of CDK inhibitors as antitumour drugs are reviewed below, with an emphasis on flavopiridol, the first CDK inhibitor to undergo clinical evaluation in humans.

Flavopiridol

Flavopiridol (NSC 649890, L86-8275 or HMR 1275) is a semisynthetic small molecular derivative of rohitukine, an alkaloid isolated from *Dysoxylum binectariferum*. Initially, flavopiridol demonstrated potent effects on cell proliferation (IC₅₀ = 66 nM) in 60 National Cancer Institute human tumour cell lines, with no obvious tumour-type selectivity [1]. This effect has also been observed in xenograft models involving various tumour-types [2]. Mechanistically, the antitumour activity of flavopiridol has been related to CDK inhibition, induction of apoptosis, inhibition of transcription and anti-angiogenic activity.

Inhibition of CDKs by flavopiridol

Cell-cycle progression (i.e. the traverse through G1, S, G2 and M phases) is tightly controlled by CDK holoenzyme complexes consisting of catalytic subunits (CDKs) and regulatory subunits (cyclins) [3]. 13 CDKs and 25 cyclin-box-containing proteins have been identified through human genome sequencing. Among these, CDKs 1, 2, 4 and 6 and cyclins A, B, D and E are directly involved in regulating progression through the cell-cycle. The CDK7/cyclinH complex (i.e. CAK) is involved in cell-cycle

regulation through phosphorylation and activation of CDKs 1, 2, 4 and 6.

Flavopiridol inhibits several protein kinases, with the greatest activity directed against CDKs. In *in vitro* and *in vivo* studies, flavopiridol has been shown to inhibit CDK1/cyclin B ($IC_{50} = 30\text{--}40\text{ nM}$), CDK2/cyclin A and CDK2/cyclin E ($IC_{50} = 100\text{ nM}$), CDK4/cyclin D ($IC_{50} = 20\text{--}40\text{ nM}$), CDK6/cyclin D ($IC_{50} = 60\text{ nM}$) and CDK7/cyclin H ($IC_{50} = 110\text{--}300\text{ nM}$) [4]. Analysis of X-ray crystal structures revealed that L868276 (a deschlorophenyl derivative of flavopiridol) binds to the ATP binding pocket of CDK2 [5]. As the molecular structures of CDKs are similar (with 40% shared homology, including the highly conserved catalytic core region of 300 residues), it is likely that flavopiridol inhibits the activity of most CDKs by directly occupying the ATP binding site, a function competitively blocked by excess ATP. Indeed, in soluble cell extracts, CDKs 1, 2, 4 and 7 bind to immobilized flavopiridol only in the absence of ATP [4]. Furthermore, flavopiridol can inhibit CDK7/cyclin H, leading to interference with phosphorylation of specific threonine residues of most CDKs (e.g. Thr161 in cdc2/CDK1, Thr160 in CDK2, Thr172 in CDK4 and Thr177 in CDK6), an event that is required for their full activation. Inhibition of CDKs 1, 2 and 4 by flavopiridol leads directly to cell-cycle arrest at the G1/S and G2/M phase transitions, and a delay in S phase progression [1,2]. In tumour cells lacking CDK4, flavopiridol induces G1 arrest by inhibiting CDK6 [4]. Downregulation of cyclin D1 (see below) can also contribute to cell-cycle arrest following flavopiridol treatment. The patterns of flavopiridol-induced cell-cycle arrest (G1/S and/or G2/M arrest) appear to be cell type-specific.

Inhibition of transcription

The elongation phase in transcription of genes encoding eukaryotic proteins is controlled by RNA polymerase II (RNAPII), and is determined by the interplay between negative (N-TEF) and positive (P-TEF) transcription elongation factors. P-TEFb is the only known component of P-TEF. Human P-TEFb is composed of CDK9 and cyclin T (T1, T2a or T2b), forming a holoenzyme complex that phosphorylates (activates) the C-terminal domain of RNAPII [6]. Specific binding of the small nuclear RNA 7SK to P-TEFb blocks CDK9 activity [7]. In addition, other CDK complexes might also participate in transcriptional regulation by either phosphorylating the C-terminal domain of RNAPII (e.g. CDK9/cyclin K, CDK7/cyclin H/Mat1 and CDK8/cyclin C), regulating pre-mRNA splicing (CDK11/cyclin L), or modulating the transcription factor Ets2 (CDK10) [8–10].

Flavopiridol potently blocks transcriptional activity of RNAPII *in vitro* by inhibiting P-TEFb (CDK9/cyclin T), a process initially related to inhibition of HIV replication ($IC_{50} < 10\text{ nM}$) [11]. Flavopiridol inhibits the C-terminal

domain kinase activity of P-TEFb (K_i of 3 nM) more potently than other CDKs (e.g. CDKs 1, 2, and 4; K_i values of 40–70 nM). Furthermore, inhibition of CDK9 by flavopiridol is non-competitive with respect to ATP [12^{••}]. Structural information indicates that flavopiridol binds to the ATP binding pocket of CDK9 with higher affinity than it does to CDK2 [13[•]]. *In vitro* and *in vivo*, flavopiridol inhibits transcription at concentrations much lower than those required for inhibition of other CDKs, even in the presence of ATP. Whether inhibition of CDK7 is involved in blocking transcription remains unknown, but it is clear that inhibition of CDK7 requires much higher concentrations of flavopiridol ($IC_{50} > 100\text{ nM}$) than those required for inhibition of transcription, at least in the case of HIV Tat (a potent transactivator of viral gene expression, which is essential for HIV-1 replication) transcription. DNA microarrays have demonstrated that flavopiridol inhibits gene expression broadly, closely mimicking the actions of two specific transcription inhibitors (actinomycin D and DRB [5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole]), indicating that flavopiridol might act as a global transcription inhibitor [14]. However, it is unclear if the predominant effect of flavopiridol on gene expression results from its ability to inhibit P-TEFb, particularly in mammalian cell systems. The potent inhibitory effects on viral gene transcription raise the possibility that CDK inhibitors (e.g. flavopiridol and roscovitine) could be developed as clinical antiviral drugs [15].

One of the most interesting molecules to be transcriptionally downregulated by flavopiridol is cyclin D1. D-type cyclins (particularly D1) are multifunctional proteins that participate in cell-cycle regulation, cell growth and carcinogenesis [16]. Exposure of breast cancer cells to flavopiridol (100–300 nM) results in a decline in cyclin D1 promoter activity, leading to a decrease in cyclin D1 mRNA and protein levels [1]. *In vivo*, flavopiridol depletes cyclin D1 in tumour xenografts [2]. Transcriptional inhibition of cyclin D1 has been associated with cell-cycle arrest and possibly cell death following flavopiridol treatment. Such observations are consistent with the finding that flavopiridol produces a significant delay in disease progression in 84% of patients with mantle cell lymphoma — a tumour with overexpression of cyclin D1 in 95% of cases [17].

Induction of apoptosis

Apoptosis plays a pivotal role in drug- and radiation-induced cytotoxicity. Mechanistically, initiation of apoptosis involves at least two distinct pathways: extrinsic (death receptor-related) and intrinsic (mitochondria-dependent) pathways. Members of the Bcl-2 family, which includes both anti-apoptotic (e.g. Bcl-2, Bcl-xL, Bcl-w, A1 and Mcl-1) and pro-apoptotic proteins (Bax family: Bax, Bak and Bok; BH3-only family: Bid, Bim, Bik, Bad, Bmf, Hrk, Noxa and Puma), are important apoptotic regulators [18]. Inhibitors of apoptosis (IAPs;

e.g. XIAP, cIAP1, cIAP2, NAIP, MLAI, ILP2, livin [KIAP], apollon and survivin) represent another regulatory protein family involved in apoptotic signaling, the members of which directly bind to and inhibit caspases 3, 6, 7 and 9 [19]. In turn, IAPs are inhibited by mitochondrial release of Smac/DIABLO (second mitochondria-derived activator of caspases/direct IAP binding protein with low pI released from mitochondria) [19].

In vitro, exposure to 100–400 nM flavopiridol for 6–48 hours induces apoptosis in a variety of tumour cells [4]. Human leukemia cells, independently of their origin and type, appear to be particularly sensitive to flavopiridol, as are multiple myeloma cells. Moreover, flavopiridol exerts lethal effects in tumour cells that are resistant to DNA-damaging agents and radiation [1]. *In vivo*, flavopiridol induces apoptosis in tumour xenografts, as detected by TUNEL assay, with significant reductions of up to 60–70% in tumour size [1]. The mechanisms responsible for flavopiridol-induced apoptosis are complex and probably multifactorial. Although cleavage of caspase-8 and Bid has been observed in tumour cells after flavopiridol treatment, it is unlikely that the extrinsic pathway represents the primary cell death trigger because flavopiridol is able to induce apoptosis in cells lacking caspase-8 [20]. Moreover, caspase-8 inhibitors (either pharmacological or viral) fail to block mitochondrial damage and apoptosis induced by flavopiridol [21].

Transcriptional inhibition of genes that encode anti-apoptotic regulators has been postulated to play a central role in the cytotoxicity of flavopiridol. For example, downregulation of Bcl-2 protein has been observed in several cell lines following flavopiridol treatment [22,23]. However, neither ectopic expression nor antisense-oligonucleotide-mediated downregulation of Bcl-2 exerts an effect on flavopiridol-induced cell death, indicating this cell death is largely independent of Bcl-2. Such findings may potentially explain why flavopiridol can overcome chemotherapeutic drug resistance secondary to Bcl-2 overexpression [20]. Interestingly, overexpression of an N-terminal phosphorylation-loop-deleted Bcl-2 protein provides a high degree of resistance to human leukemia cells treated with flavopiridol [24].

Recently, evidence has appeared suggesting that the anti-apoptotic protein Mcl-1 represents an important flavopiridol target. Firstly, decreases in Mcl-1 protein and/or mRNA levels have been observed in flavopiridol-treated tumour cell lines (e.g. lung cancer and multiple myeloma), freshly isolated tumour cells (i.e. from patients with B cell chronic lymphocytic leukemia and multiple myeloma) and in leukemic cells obtained from flavopiridol-treated patients [25,26^{••},27–29]. Secondly, downregulation of Mcl-1 has been related to accumulation of E2F-1 [28], disruption of signal transducer and activator of transcription-3 (STAT3)/DNA binding [29]

and direct inhibition of C-terminal domain phosphorylation of RNAPII associated with flavopiridol treatment [26^{••}]. Moreover, enforced expression of Mcl-1 provides a high degree of resistance to tumour cells against flavopiridol toxicity [26^{••},28]. In addition, downregulation of other anti-apoptotic proteins, including XIAP, BAG-1 and Bcl-xL, has been reported in certain tumour cells treated *in vitro* with flavopiridol [25,30].

Flavopiridol can also directly bind to duplex DNA [31], a finding that provides a possible explanation for the capacity of flavopiridol to kill noncycling (resting) cancer cells, as well as normal cells (e.g. peripheral lymphocytes, endothelial cells and embryonic neurons). However, normal cells appear to be less sensitive than tumour cells to flavopiridol-mediated lethality. It is unclear whether inhibition of CDK-mediated pathways is required for induction of apoptosis. In addition, there is a lack of evidence demonstrating that transcriptional repression of cyclin D1 by flavopiridol contributes functionally to its cytotoxicity, although antisense-mediated reductions in cyclin D1 expression can induce apoptosis in carcinoma cells [4].

Interestingly, administration of flavopiridol dramatically protects neurons from cell death induced by a variety of pathological stimuli. This phenomenon appears to be related to inhibition of CDK activity (CDK4/cyclin D1 and probably CDKs 1, 2 and 6) [32–34].

Synergism between flavopiridol and conventional cytotoxic agents

Cotreatment with flavopiridol and cytostatic drugs significantly increases cytotoxicity in a variety of tumour cells. In most cases, synergistic interactions are sequence-dependent, with administration of cytostatic drugs before flavopiridol yielding optimal lethality. Following the success of *in vitro* preclinical models, several clinical trials combining flavopiridol and various standard chemotherapeutic agents have been initiated (see below) [35,36]. The mechanisms underlying synergism appear to differ depending upon the cytostatic drugs used. Exposure of tumour cells to adriamycin and taxol results in a four- to five-fold increase in expression of survivin, whereas inhibition of p34cdc2/cyclinB-mediated survivin phosphorylation on Thr34 by flavopiridol results in diminished expression of survivin [37^{••}]. These findings might explain why sequential administration of flavopiridol after such agents enhances apoptosis. In lung cancer cells, pretreatment with non-toxic concentrations of gemcitabine or cisplatin imposes an S-phase delay, which renders transformed cells selectively sensitive to the cytotoxicity of flavopiridol [38^{••}]. Conversely, pre-treatment with flavopiridol can recruit leukemia cells into a proliferative state and thus 'prime' them for the S-phase-related cytotoxicity of nucleoside analogues such as ara-C [39]. Synergism following sequential treatment of tumour cells with gemcitabine and then flavopiridol might also be related to

ubiquitin/proteasome-dependent degradation of E2F-1, resulting in downregulation of the ribonucleotide reductase M2 subunit, which has been shown to contribute to gemcitabine resistance [40]. Flavopiridol selectively enhances doxorubicin-mediated cell death in cells exhibiting a functional defect in pRb, which is a common abnormality in human cancers [41]. Apoptosis induced by treatment with SN-38 (the active metabolite of camptothecin CPT-11) followed by flavopiridol is associated with cleavage of p21^{cip1} and XIAP, and suppression of Drg1 expression, a novel gene responsible for resistance to CPT-11 [42,43]. Finally, recent studies suggest that sequence-dependent cytotoxicity of Epo-B (a non-taxane tubulin-polymerizing agent) followed by flavopiridol may be related to flavopiridol-induced Bax conformational change and downregulation of IAPs (XIAP, cIAP2 and survivin), Bcl-xL and Mcl-1 [44].

Synergism between flavopiridol and novel agents

There is increasing evidence that synergistic interactions also exist between flavopiridol and a variety of other novel signaling modulators. One interesting finding is that flavopiridol appears to convert drug-induced cell differentiation into apoptosis [45]. For example, simultaneous, but not sequential, exposure of leukemia cells to subtoxic concentrations of flavopiridol and the phorbol ester PMA (a potent inducer of differentiation) synergistically induces apoptosis and overcomes resistance mediated by overexpression of Bcl-2 [46^{*}]. Such synergism is associated with abrogation of PMA-induced p21^{cip1} expression by flavopiridol. These findings have now been extended to include other classes of differentiation-inducing agents, including inhibitors of histone deacetylase, such as suberoylanilide hydroxamic acid and sodium butyrate. Thus, cotreatment with flavopiridol blocks p21^{cip1} induction by butyrate and suberoylanilide hydroxamic acid, leading to synergistic induction of apoptosis in multiple leukemia cell-types [47]. Such findings are consistent with earlier reports indicating that antisense-mediated inhibition of p21^{cip1} expression blocks differentiation and increases leukaemic cell sensitivity to butyrate-mediated apoptosis [48]. In addition, synergistic interactions between flavopiridol and PMA or histone deacetylase inhibitors have been associated with downregulation of cyclin D1 and Mcl-1, cleavage of pRb and Bcl-2, and activation of E2F-1. Subsequent studies demonstrated that synergism between flavopiridol and PMA stemmed from PKC-dependent induction of tumour necrosis factor- α and activation of the extrinsic apoptotic pathway [49]. Analogously, the protein kinase C activator bryostatins has also been shown to interact synergistically with flavopiridol through a similar tumour necrosis factor- α -related mechanism [50]. Consistent with this model, recent evidence has appeared indicating that flavopiridol interacts synergistically with TRAIL (TNF-related apoptosis-inducing ligand) [51]. Flavopiridol has also been shown to potentiate STI571-induced apoptosis

in Bcr-Abl-positive leukemia cells, including those resistant to the Bcr/Abl kinase inhibitor STI571 (Gleevec, imatinib mesylate) [52]. Interestingly, synergism between flavopiridol and STI571 was associated with downregulation of Mcl-1, Bcl-xL and cyclin D1, and activation of Jun N-terminal kinase. Flavopiridol and herceptin (trastuzumab, anti-erbB2 antibody) interact synergistically in breast cancer cells in association with both inhibition of the Ras/MAPK/Akt pathway and diminished cyclin D1 expression [53,54]. Finally, cytotoxic synergy has been observed between flavopiridol and LY294002, a selective phosphatidylinositol 3-kinase inhibitor [54], suggesting an important role for the phosphatidylinositol 3-kinase/Akt pathway in protecting leukaemic cells from flavopiridol-mediated lethality. Collectively, these findings suggest that the concept of combining flavopiridol with other novel signaling modulators warrants further exploration as an anti-cancer strategy.

Other potential mechanisms of flavopiridol lethality

In several systems, flavopiridol has shown significant anti-angiogenic activity. Such activity has been related to the ability of flavopiridol to induce apoptosis in endothelial cells through an unknown mechanism, which appears to be independent of CDK inhibition [4]. Another possibility is that flavopiridol acts through inhibition of vascular endothelial growth factor (VEGF) expression. For example, it has been shown that flavopiridol blocks hypoxia-induced VEGF mRNA transcription and decreases VEGF mRNA stability, leading to downregulation of VEGF protein [55].

Glycogen phosphorylase has been identified as a flavopiridol-binding protein [56]. Such direct binding could lead to the inhibition of glycogen phosphorylase. Indeed, flavopiridol treatment causes an increase in glycogen accumulation in lung cancer cells [57]. These findings raise the possibility that interference with glucose homeostasis may also contribute to the antineoplastic effects of flavopiridol.

Flavopiridol resistance

Resistance of tumour cells to chemotherapeutic drugs represents a major obstacle in anti-cancer therapy. It has been reported that flavopiridol fails to exhibit cross-resistance to most conventional cytotoxic agents, including etoposide, doxorubicin, vinblastine, oxaliplatin, paclitaxel, cisplatin, topotecan and 5FU [4]. More specifically, overexpression of *mdr-1* (encoding PgP-170) or *mrp-1* (encoding MRP-190), the major multidrug resistance proteins implicated in conferring resistance to natural product cytotoxic agents, does not cause appreciable resistance to flavopiridol [58–60]. Thus, the lack of cross-resistance between conventional cytotoxic agents and flavopiridol provides a basis for developing combination regimens incorporating flavopiridol and established cytotoxic agents.

Clinical trials

Phase I and II trials of flavopiridol have been completed involving two major schedules (i.e. a 72-hour infusion every two weeks, or a one hour infusion for five, three or one day every three weeks). In the 72-hour infusion regimen, steady-state plasma flavopiridol concentrations were 271–415 nM [1,2,35] whereas, in the one hour infusion regimen, the median post-infusion peak concentration was 1.7–3.8 μ M [1,2,35]. These clinically achievable concentrations were significantly above those required to inhibit CDKs and cell growth, and induce apoptosis *in vitro*. In addition, there was evidence of antitumour activity against renal cell carcinoma, colon carcinoma, non-Hodgkin lymphoma, gastric carcinoma and especially mantle cell lymphoma [1,17]. However, the activity of flavopiridol administered alone has been less than that encountered in *in vitro* and xenograft model systems. Therefore, in addition to further phase I and II single-drug trials, efforts are underway to combine flavopiridol with other FDA-approved anti-cancer agents, including paclitaxel, fludarabine, cytosine arabinoside and irinotecan [35,36]. Attempts to combine flavopiridol with other signal transduction modulators are also under development.

Other CDK inhibitors

As a member of the first generation of CDK inhibitors, flavopiridol acts as a 'pan' CDK antagonist and, as such, is not particularly selective for a specific CDK. However, more selective CDK inhibitors have since been developed. Several of these compounds are being evaluated for antitumour activity in preclinical model systems.

Purine-based CDK inhibitors display greater selectivity for CDKs 1, 2 and probably 5, but exhibit no inhibitory activity toward CDKs 4 and 6. Some of these inhibitors have been reported to exert potent antitumour activity, including olomoucine, roscovitine, purvalanols and CGP74514A (a 2,6,9-trisubstituted purine).

Olomoucine and roscovitine

Olomoucine was one of the first CDK inhibitors to be developed, and selectively inhibits CDKs 1, 2, 5 and probably 7 (but not CDK4) at micromolar concentrations. It has been shown to exert 50% growth inhibition against a National Cancer Institute panel of 60 tumour cell lines ($IC_{50} = 60.3 \mu$ M), typically arresting cells at the G1/S and G2/M transitions [5]. Roscovitine is an olomoucine derivative and shares similar selectivity for CDKs (i.e. CDKs 1, 2 and 5, but not CDKs 4 and 6). However, it displays more potent (5- to 10-fold greater) inhibitory activity towards CDKs (particularly CDK1), and increased antitumour activity (average $IC_{50} = 16 \mu$ M for all 60 tumour cell lines) [5]. *In vitro*, roscovitine also inhibits CDK3, which is involved in cell-cycle regulation [61]. Roscovitine and olomoucine can also efficiently stabilize and activate nuclear p53 by suppressing MDM2 (murine

double minute 2) expression, which might permit them to function as sensitizing agents for DNA-damaging drugs [62,63]. Higher concentrations of roscovitine can block synthesis of RNA (probably by inhibiting phosphorylation of the C-terminal domain of RNAPII in a similar manner to flavopiridol) and DNA (by direct effects on DNA synthesis machinery) [63,64].

Interestingly, purine-based CDK inhibitors, such as roscovitine, can also exert anti-apoptotic effects in the nervous system [33,65], where activation and/or subcellular translocation (from nuclei to cytoplasm) of CDKs (e.g. CDKs 2, 4 and 5) appears to be essential for induction of cell death. Moreover, roscovitine can prevent neuronal death in neurodegenerative diseases (e.g. Alzheimer's disease) [66,67], possibly by inhibiting CDK5/p25-catalysed phosphorylation of the tau protein and/or CDK1-mediated phosphorylation and extracellular accumulation of β -amyloid, which are both hallmarks of these diseases.

CYC202

CYC202 (*R*-roscovitine) has been reported to display increased potency compared with roscovitine, with CYC202 showing more selective inhibition of CDK2/cyclin E ($IC_{50} = 100$ nM) and enhanced antitumour activity ($IC_{50} = 15.2 \mu$ M in a panel of 19 human tumour cell lines) [68]. Indeed, in xenograft animal tumour models, CYC202 has been shown to exert significant antitumour effects. Specifically, it has been shown to induce cell death rather than cell-cycle arrest, with a 45–62% reduction in tumour growth [68]. Phase I trials of CYC202 are currently underway, making this the second CDK inhibitor to enter the clinical arena.

Purvalanols

Purvalanol A and B, which are derivatives of olomoucine, exhibit potent and selective inhibition of CDKs 1 and 2 with 1000-fold greater inhibitory activity toward CDK2/cyclin A compared with olomoucine [5]. *In vitro* studies demonstrated that purvalanol inhibits cell growth ($IC_{50} = 2.5 \mu$ M) and induces G2/M arrest. These effects are linked to inhibition of CDK1 and p42/p44 mitogen-activated protein kinase, both of which have been identified as intracellular targets of purvalanol [69]. Administration of purvalanol did not appear to trigger extensive activation of caspases; however, induction of apoptosis might be highly dependent upon the concentration of CDK inhibitors. For example, it has been shown that treatment of leukemia cells with low concentrations (1 μ M) of the tri-substituted purine CGP74514A induces cell-cycle arrest, whereas higher concentrations (> 3 μ M) trigger mitochondrial damage and cell death [70]. Similarly, analogues of olomoucine, including plant cytokinin analogues, exhibited either cell-cycle inhibitory effects or induction of apoptosis in a dose-dependent manner in leukemia cells [71].

Indolinones

Indolinone-based CDK inhibitors also selectively inhibit CDKs 1, 2 and 5. For example, SU9516, initially developed as a CDK2-selective inhibitor, displayed more potent inhibition of CDK2 ($IC_{50} = 22$ nM) and CDK1 ($IC_{50} = 40$ nM) than of CDK4 ($IC_{50} = 200$ nM). In colon carcinoma cells, administration of SU9516 resulted in inhibition of CDK2 and diminished pRb phosphorylation, accompanied by either G0/G1 or G2/M arrest and caspase-3-mediated apoptosis [72].

Paullones

The paullone class of CDK inhibitors displays a high degree of selectivity for CDKs 1, 2 and 5, but no activity towards CDK4/cyclin D1. Thus, the inhibitory spectrum of this class of CDK inhibitors is similar to that of purine-based compounds. Among members of this class, kenpaullone and its derivative alsterpaullone have been found to be the most active against CDK1/cyclin B and are competitive with ATP [73]. The antitumour potential of the paullone class CDK inhibitors remains to be evaluated.

UCN-01

UCN-01 (7-hydroxystaurosporine) was originally developed as a selective protein kinase C inhibitor. However, it was subsequently shown that at higher ($IC_{50} = 300$ – 600 nM) concentrations UCN-01 inhibits CDK1 (cdc2) and CDK2 *in vitro* [1], and therefore can be characterized as a CDK inhibitor. Nevertheless, other studies have shown that the antitumour activity of UCN-01 is primarily related to its ability to induce abrogation of the G2 or G1 checkpoints ($IC_{50} \sim 50$ nM), resulting in 'inappropriate' activation of CDKs (e.g. cdc2/CDK1) [1,74]. This appears to represent a consequence of inhibition of phosphorylation of Cdc25C by the kinase chk1 [75]. Interference with the G1 and G2 checkpoints appears to be responsible for synergistic interactions between UCN-01 and conventional cytotoxic agents (e.g. cisplatin, ara-C and gemcitabine) [76–78]. More recently, UCN-01 has been shown to inhibit Akt [79], although the contribution of this action to the antitumour activity of UCN-01 is unclear. Clinical trials of UCN-01, either alone or in combination with established cytotoxic drugs, have begun in humans, and have revealed an exceptionally long plasma half-life for the compound as a result of extensive binding to plasma α 1-acidic glycoprotein [80].

The search for selective CDK inhibitors

CDK4/cyclin D represents a desirable target for drug discovery for a variety of reasons, including its frequent dysregulation in human neoplasia. However, the development of selective CDK4 inhibitors is more difficult than that of CDK2 inhibitors, primarily because the crystal structure of CDK4 has not been acquired. Nevertheless, CDK inhibitors derived from pyrimidines have been found to selectively inhibit CDKs 4 and 6. For example, PD0183812 has been identified as a highly

selective CDK 4 and 6 inhibitor ($IC_{50} = 8$ nM for CDK4/cyclin D1; 7.1 nM for CDK6/cyclin D2; and 13 nM for CDK6/cyclin D3), which induces G1 arrest and blocks proliferation only in tumour cells expressing pRb, resulting in a loss of pRb phosphorylation [81]. CINK4, a triaminopyrimidine derivative, is another pyrimidine-based compound that specifically inhibits CDK4/cyclin D1 ($IC_{50} = 1.5$ μ M) *in vitro*. It has been shown that CINK4 causes growth arrest at the G1 phase in tumour cells and normal cells, and slows tumour growth in xenografts of colon carcinoma cells [82].

Although no inhibitor has been identified that is absolutely selective for a single CDK, the search continues for natural products and structure-based synthetic or semi-synthetic compounds that will selectively target particular CDKs (e.g. CDKs 1, 2 and 4) [83]. However, because of the conservation of amino acids framing the CDK ATP-binding pocket and the high structural homology in the CDK family, it might be difficult to design single CDK-specific inhibitors. In this regard, three-dimensional structural models of CDKs should provide useful information for the design of novel CDK-selective inhibitors. For example, the crystal structure of CDK2 is well characterized and has been widely used for the development of CDK2-specific inhibitors. As a result, a new purine-based CDK inhibitor has been described that is 1000-fold more potent than the parent compound ($K_i = 9$ nM for CDK1, and 6 nM for CDK2) [84^{*}]. Similar efforts are underway to develop CDK4-specific inhibitors by using a CDK4 mimic CDK2 protein [85]. Alternatively, new approaches, including affinity chromatography utilizing immobilized inhibitors, have been employed to characterize the intracellular targets and selectivity of a particular CDK inhibitor [86^{*}]. One question that remains to be resolved is whether a high degree of selectivity for a particular CDK represents a desirable characteristic for CDK inhibitors designed to function as antineoplastic agents.

Conclusions

Recently, we have witnessed a tremendous increase in our understanding of the factors regulating the cell-cycle, the role of cell-cycle dysregulation in carcinogenesis and the relationship between cell-cycle events and apoptosis. These have, in turn, served as a powerful impetus for the development of CDK inhibitors as antineoplastic agents. To date, the CDK inhibitors flavopiridol and CYC202 have entered the clinical arena, and others are certain to follow. However, several issues remain to be resolved before such agents can fulfill their potential. These include defining the relationship between CDK inhibition and induction of apoptosis, determining whether selective inhibition of a particular CDK is a desirable goal, and optimizing the use of CDK inhibitors in combination with other agents, including both conventional and novel antineoplastic drugs. Given the accelerated pace

of discovery in the area of cell-cycle regulation, it is apparent that the development of CDK inhibitors will be the subject of intense interest for many years to come.

Acknowledgements

This work was supported by awards CA6375, CA83705, CA93738 and CA100866 from the National Cancer Institute, and award 6045-03 from the Leukemia and Lymphoma Society of America.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Senderowicz AM: **The cell cycle as a target for cancer therapy: basic and clinical findings with the small molecule inhibitors flavopiridol and UCN-01.** *Oncologists* 2002, **7**:12-19.
 2. Zhai S, Senderowicz AM, Sausville EA, Figg WD: **Flavopiridol, a novel cyclin-dependent kinase inhibitor, in clinical development.** *Ann Pharmacol* 2002, **36**:905-911.
 3. Sandal T: **Molecular aspects of the mammalian cell cycle and cancer.** *Oncologists* 2002, **7**:73-81.
 4. Sedlacek HH: **Mechanisms of action of flavopiridol.** *Cri Rev Oncol Hematol* 2001, **38**:139-170.
 5. Hardcastle IR, Golding BT, Griffin RJ: **Designing inhibitors of cyclin-dependent kinases.** *Annu Rev Pharmacol Toxicol* 2002, **42**:325-348.
 6. Shim EY, Walker AK, Shi Y, Blackwell TK: **CDK-9/cyclin T (P-TEFb) is required in two postinitiation pathways for transcription in the *C. elegans* embryo.** *Genes Dev* 2002, **16**:2135-2146.
 7. Nguyen VT, Kiss T, Michels AA, Bensaude O: **7SK small nuclear RNA binds to and inhibits the activity of CDK9/cyclin T complex.** *Nature* 2001, **414**:322-325.
 8. Coqueret O: **Linking cyclins to transcriptional control.** *Gene* 2002, **299**:35-55.
 9. Wallenfang MR, Seydoux G: **Cdk7 is required for mRNA transcription and cell cycle progression in *Caenorhabditis elegans* embryos.** *Proc Natl Acad Sci USA* 2002, **99**:5527-5532.
 10. Hu D, Mayeda A, Trembley JH, Lahti JM, Kidd VJ: **CDK11 complexes promote pre-mRNA splicing.** *J Biol Chem* 2003, **278**:8623-8629.
 11. Chao SH, Fujinaga K, Marion JE, Taube R, Sausville EA, Senderowicz AM, Matija Peterlin B, Price DH: **Flavopiridol inhibits P-TEFb and blocks HIV-1 replication.** *J Biol Chem* 2000, **275**:28345-28348.
 12. Chao SH, Price DH: **Flavopiridol inactivates P-TEFb and blocks •• most RNA polymerase II transcription *in vivo*.** *J Biol Chem* 2001, **276**:31793-31799.
- The authors first noted that flavopiridol could inhibit P-TEFb (CDK9/cyclin T) in HIV-1 replication [11] and extended this finding to include most RNAPII transcription *in vivo*. This constituted the first demonstration that flavopiridol could function as a transcriptional repressor.
13. de Azevedo WF, Canduri F, da Silveira NJF: **Structural basis for • inhibition of cyclin-dependent kinase 9 by flavopiridol.** *Biochem Biophys Res Commun* 2002, **293**:566-571.
- Crystal structural analysis demonstrated that flavopiridol inhibits CDK9 by direct binding to its ATP-binding pocket. These data provide a clear basis for understanding the nature of CDK9 inhibition by flavopiridol.
14. Lam LT, Pickeral OK, Peng AC, Rosenwald A, Hurt EM, Giltman JM, Averett LM, Zhao H, Davis RE, Sathiyamoorthy M *et al.*: **Genomic-scale measurement of mRNA turnover and the mechanisms of action of the anti-cancer drug flavopiridol.** *Genome Biol* 2001, **2**:RESEARCH0041.
 15. Schang LM: **Cyclin-dependent kinases as cellular targets for antiviral drugs.** *J Antimicrob Chemother* 2002, **50**:779-792.
 16. Ortega S, Malumbres M, Barbacid M: **Cyclin D-dependent kinases, INK4 inhibitors and cancer.** *Biochim Biophys Acta* 2002, **1602**:73-87.
 17. Sausville EA, Elsayed Y, Monga M, Kim G: **Signal transduction-directed cancer treatments.** *Annu Rev Pharmacol Toxicol* 2003, **43**:199-231.
 18. Cory S, Adams JM: **The Bcl-2 family: regulators of the cellular life-or-death switch.** *Nat Rev Cancer* 2002, **2**:647-656.
 19. Igney FH, Krammer PH: **Death and anti-death: tumor resistance to apoptosis.** *Nat Rev Cancer* 2002, **2**:277-288.
 20. Achenbach TV, Muller R, Slater EP: **Bcl-2 independence of flavopiridol-induced apoptosis.** *J Biol Chem* 2000, **275**:32089-32097.
 21. Decker RH, Dai Y, Grant S: **The cyclin-dependent kinase inhibitor flavopiridol induces apoptosis in human leukemia cells (U937) through the mitochondrial rather than the receptor-mediated pathway.** *Cell Death Differ* 2001, **8**:715-724.
 22. Pepper C, Thomas A, Hoy T, Fegan C, Bentley P: **Flavopiridol circumvents Bcl-2 family mediated inhibition of apoptosis and drug resistance in B-cell chronic lymphocytic leukemia.** *Br J Haematol* 2001, **114**:70-77.
 23. Li Y, Chinni SR, Senderowicz AM, Sarkar FH: **Induction of growth inhibition and apoptosis in prostate cancer cells by flavopiridol.** *Int J Oncol* 2000, **17**:755-759.
 24. Decker RH, Wang S, Dai Y, Grant S: **Loss of the Bcl-2 phosphorylation loop domain is required to protect human myeloid leukemia cells from flavopiridol-mediated mitochondrial damage and apoptosis.** *Cancer Biol Ther* 2002, **1**:136-144.
 25. Kitada S, Zapata JM, Andreeff M, Reed JC: **Protein kinase inhibitors flavopiridol and 7-hydroxy-staurosporine down-regulate antiapoptotic proteins in B-cell chronic lymphocytic leukemia.** *Blood* 2000, **96**:393-397.
 26. Gojo I, Zhang B, Fenton RG: **The cyclin-dependent kinase •• inhibitor flavopiridol induces apoptosis in multiple myeloma cells through transcriptional repression and down-regulation of Mcl-1.** *Clin Cancer Res* 2002, **8**:3527-3538.
- An important survival factor, Mcl-1, was shown to be transcriptionally downregulated by flavopiridol, most likely through inhibition of RNAPII. This observation establishes a relationship between transcriptional inhibition and the lethality of flavopiridol.
27. Semenov I, Akyuz C, Roginskaya V, Chauhan D, Corey SJ: **Growth inhibition and apoptosis of myeloma cells by the CDK inhibitor flavopiridol.** *Leuk Res* 2002, **26**:271-280.
 28. Ma Y, Cress D, Haura EB: **Flavopiridol-induced apoptosis is mediated through up-regulation of E2F1 and repression of Mcl-1.** *Mol Cancer Ther* 2003, **2**:73-81.
 29. Lee YK, Tidwell ML, Kaufmann SH, Karp JE, Bible KC: **Flavopiridol disrupts STAT-3/DNA binding and down-regulates the downstream antiapoptotic protein MCL-1 *in vivo* in leukemia cells from flavopiridol-treated AML patients.** *Proc Am Assoc Cancer Res* 2002, **43**:127.
 30. Mihara M, Shintani S, Nakashiro K, Hamakawa H: **Flavopiridol, a cyclin-dependent kinase (CDK) inhibitor, induces apoptosis by regulating Bcl-x in oral cancer cells.** *Oral Oncol* 2003, **39**:49-55.
 31. Bible KC, Bible RH, Kottke TJ, Svingen PA, Xu K, Pang Y, Hajdu E, Kaufmann SH: **Flavopiridol binds to duplex DNA.** *Cancer Res* 2000, **60**:2419-2428.
 32. Osuga H, Osuga S, Wang F, Fetni R, Hogan MJ, Slack RS, Hakim AM, Ikeda J-E, Park DS: **Cyclin-dependent kinases as a therapeutic target for stroke.** *Proc Natl Acad Sci USA* 2000, **97**:10254-10259.
 33. O'Hare M, Wang F, Park DS: **Cyclin-dependent kinases as potential targets to improve stroke outcome.** *Pharmacol Ther* 2002, **93**:135-143.
 34. Ghahremani MH, Keramaris E, Shree T, Xia Z, Davis RJ, Flavell R, Slack RS, Park DS: **Interaction of the c-Jun/JNK pathway and cyclin-dependent kinases in death of embryonic cortical neurons evoked by DNA damage.** *J Biol Chem* 2002, **277**:35586-35596.

35. Colevas D, Blaylock B, Gravell A: **Current clinical trials of flavopiridol.** *Oncology* 2002, **16**:1204-1214.
36. Schwartz GK, O'Reilly E, Ilson D, Saltz L, Sharma S, Tong W, Maslak P, Stoltz M, Eden L, Perkins P *et al.*: **Phase I study of the cyclin-dependent kinase inhibitor flavopiridol in combination with paclitaxel in patients with advanced solid tumors.** *J Clin Oncol* 2002, **20**:2157-2170.
37. Wall NR, O'Connor DS, Plescia J, Pommier Y, Altieri DC: **Suppression of survivin phosphorylation on Thr34 by flavopiridol enhances tumor cell apoptosis.** *Cancer Res* 2003, **63**:230-235.
Inhibition of cdc2/CDK1-mediated phosphorylation by flavopiridol diminished the increased expression of the anti-apoptotic protein survivin induced by the cytostatic drugs adriamycin and taxol. This finding might explain the sequence-dependent nature of synergism between flavopiridol and certain cytostatic drugs, and may also provide a basis for understanding synergistic interactions between flavopiridol and conventional cytotoxic agents.
38. Matranga CB, Shapiro GI: **Selective sensitization of transformed cells to flavopiridol-induced apoptosis following recruitment to S-phase.** *Cancer Res* 2002, **62**:1707-1717.
Recruitment to S-phase, either by synchronization or by treatment with noncytotoxic doses of chemotherapeutic agents, maximized sensitivity to flavopiridol, a phenomenon that is very selective for transformed cells. This finding might explain sequence-dependent cytotoxic synergy between conventional chemotherapy drugs and flavopiridol.
39. Karp JE, Ross DD, Yang W, Tidwell ML, Wei Y, Greer J, Mann DL, Nakasishi T, Wright JJ, Colevas AD: **Timed sequential therapy of acute leukemia with flavopiridol: *in vitro* model for a phase I clinical trial.** *Clin Cancer Res* 2003, **9**:307-315.
40. Jung CP, Motwani MV, Schwartz GK: **Flavopiridol increases sensitization to gemcitabine in human gastrointestinal cancer cell lines and correlates with down-regulation of ribonucleotide reductase M2 subunit.** *Clin Cancer Res* 2001, **7**:2527-2536.
41. Li W, Fan J, Bertino JR: **Selective sensitization of retinoblastoma protein-deficient sarcoma cells to doxorubicin by flavopiridol-mediated inhibition of cyclin-dependent kinase 2 kinase activity.** *Cancer Res* 2001, **61**:2579-2582.
42. Motwani M, Jung C, Sirotiak FM, She Y, Shah MA, Gonen M, Schwartz GK: **Augmentation of apoptosis and tumor regression by flavopiridol in the presence of CPT-11 in Hct116 colon cancer monolayers and xenografts.** *Clin Cancer Res* 2001, **7**:4209-4219.
43. Motwani M, Sirotiak FM, She Y, Commes T, Schwartz GK: **Drg1, a novel target for modulating sensitivity to CPT-11 in colon cancer cells.** *Cancer Res* 2002, **62**:3950-3955.
44. Wittmann S, Bali P, Donapathy S, Nimmanapalli R, Gao F, Yamaguchi H, Huang M, Jove R, Wang HG, Bhalla K: **Flavopiridol down-regulates antiapoptotic proteins and sensitizes human breast cancer cells to epothilone B-induced apoptosis.** *Cancer Res* 2003, **63**:93-99.
45. Grant S, Dent P: **Conversion of drug-induced differentiation to apoptosis by pharmacologic cyclin-dependent kinase inhibitors.** *Cell Cycle* 2002, **1**:383-388.
46. Cartee L, Wang Z, Decker RH, Chellappan SP, Fusaro G, Hirsch KG, Sankala HM, Dent P, Grant S: **The cyclin-dependent kinase inhibitor (CDKI) flavopiridol disrupts phorbol 12-myristate 13-acetate-induced differentiation and CDKI expression while enhancing apoptosis in human myeloid leukemia cells.** *Cancer Res* 2001, **61**:2583-2591.
This study demonstrated that flavopiridol blocked PMA-induced p21^{cip1} expression and leukemic cell maturation, while potentiating apoptosis. This finding indicates that flavopiridol can convert differentiation agents into inducers of apoptosis (also see [47,48]), and that combinational regimens involving flavopiridol and novel signal transduction modulators may represent a novel antineoplastic strategy.
47. Almenara J, Rosato R, Grant S: **Synergistic induction of mitochondrial damage and apoptosis in human leukemia cells by flavopiridol and the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA).** *Leukemia* 2002, **16**:1331-1343.
48. Rosato RR, Almenara JA, Cartee L, Betts V, Chellappan SP, Grant S: **The cyclin-dependent kinase inhibitor flavopiridol disrupts sodium butyrate-induced p21WAF1/CIP1 expression and maturation while reciprocally potentiating apoptosis in human leukemia cells.** *Mol Cancer Ther* 2002, **1**:253-266.
49. Cartee L, Smith R, Dai Y, Rahmani M, Rosato R, Almenara J, Dent P, Grant S: **Synergistic induction of apoptosis in human myeloid leukemia cells by phorbol 12-myristate 13-acetate and flavopiridol proceeds via activation of both the intrinsic and tumor necrosis factor-mediated extrinsic cell death pathways.** *Mol Pharmacol* 2002, **61**:1313-1321.
50. Cartee L, Maggio SC, Smith R, Sankala HM, Dent P, Grant S: **Protein kinase C-dependent activation of the tumor necrosis factor receptor-mediated extrinsic cell death pathway underlies enhanced apoptosis in human myeloid leukemia cells exposed to bryostatin 1 and flavopiridol.** *Mol Cancer Ther* 2003, **2**:83-93.
51. Kim DM, Koo SY, Jeon K, Kim MH, Lee J, Hong CY, Jeong S: **Rapid induction of apoptosis by combination of flavopiridol and tumor necrosis factor (TNF)-alpha or TNF-related apoptosis-inducing ligand in human cancer cell lines.** *Cancer Res* 2003, **63**:621-626.
52. Yu C, Krystal G, Dent P, Grant S: **Flavopiridol potentiates ST1571-induced mitochondrial damage and apoptosis in BCR-ABL-positive human leukemia cells.** *Clin Cancer Res* 2002, **8**:2976-2984.
53. Nahta R, Iglehart JD, Kempkes B, Schmidt EV: **Rate-limiting effects of cyclin D1 in transformation by ErbB2 predicts synergy between herceptin and flavopiridol.** *Cancer Res* 2002, **62**:2267-2271.
54. Wu K, Wang C, D'Amico M, Lee RJ, Albanese C, Pestell RG, Mani S: **Flavopiridol and trastuzumab synergistically inhibit proliferation of breast cancer cells: association with selective cooperative inhibition of cyclin D1-dependent kinase and Akt signaling pathways.** *Mol Cancer Ther* 2002, **1**:695-706.
55. Rapella A, Negrioli A, Melillo G, Pastorino S, Varesio L, Bosco MC: **Flavopiridol inhibits vascular endothelial growth factor production induced by hypoxia or picolinic acid in human neuroblastoma.** *Int J Cancer* 2002, **99**:658-664.
56. Oikonomakos NG, Schnier JB, Zographos SE, Skamnaki VT, Tsitsanou KE, Johnson LN: **Flavopiridol inhibits glycogen phosphorylase by binding at the inhibitor site.** *J Biol Chem* 2000, **275**:34566-34573.
57. Kaiser A, Nishi K, Gorin FA, Walsh DA, Bradbury EM, Schnier JB: **The cyclin-dependent kinase (CDK) inhibitor flavopiridol inhibits glycogen phosphorylase.** *Arch Biochem Biophys* 2001, **386**:179-187.
58. Robey RW, Medina-Perez WY, Nishiyama K, Lahusen T, Miyake K, Litman T, Senderowicz AM, Ross DD, Bates SE: **Overexpression of the ATP-binding cassette half-transporter, ABCG2 (Mxr/BCRP/ABCP1), in flavopiridol-resistant human breast cancer cells.** *Clin Cancer Res* 2001, **7**:145-152.
59. Smith V, Raynaud F, Workman P, Kelland LR: **Characterization of a human colorectal carcinoma cell line with acquired resistance to flavopiridol.** *Mol Pharmacol* 2001, **60**:885-893.
60. Boerner SA, Tourne ME, Kaufmann SH, Bible KC: **Effect of P-glycoprotein on flavopiridol sensitivity.** *Br J Cancer* 2001, **84**:1391-1396.
61. Keezer SM, Gilbert DM: **Evidence for a pre-restriction point Cdk3 activity.** *J Cell Biochem* 2002, **85**:545-552.
62. Lu W, Chen L, Peng Y, Chen J: **Activation of p53 by roscovitine-mediated suppression of MDM2 expression.** *Oncogene* 2001, **20**:3206-3216.
63. Ljungman M, Paulsen MT: **The cyclin-dependent kinase inhibitor roscovitine inhibits RNA synthesis and triggers nuclear accumulation of p53 that is unmodified at Ser15 and Lys382.** *Mol Pharmacol* 2001, **60**:785-789.
64. Vitali L, Yakisich JS, Vita MF, Fernandez A, Settembrini L, Siden A, Cruz M, Carminatti H, Casas O, Idoyaga Vargas V: **Roscovitine inhibits ongoing DNA synthesis in human cervical cancer.** *Cancer Lett* 2002, **180**:7-12.

65. Hiromura K, Pippin JW, Blonski MJ, Roberts JM, Shankland SJ: **The subcellular localization of cyclin dependent kinase 2 determines the fate of mesangial cells: role in apoptosis and proliferation.** *Oncogene* 2002, **21**:1750-1758.
 66. Filgueira de Azevedo W Jr, Gaspar RT, Canduri F, Camera JC Jr, Freitas da Silveira NJ: **Molecular model of cyclin-dependent kinase 5 complexed with roscovitine.** *Biochem Biophys Res Commun* 2002, **297**:1154-1158.
 67. Knockaert M, Greengard P, Meijer L: **Pharmacological inhibitors of cyclin-dependent kinases.** *Trends Pharmacol Sci* 2002, **23**:417-425.
 68. McClue SJ, Blake D, Clarke R, Cowan A, Cummings L, Fischer PM, MacKenzie M, Melville J, Stewart K, Wang S *et al.*: ***In vitro* and in vivo antitumor properties of the cyclin dependent kinase inhibitor CYC202 (R-roscovitine).** *Int J Cancer* 2002, **102**:463-468.
 69. Knockaert M, Lenormand P, Gray N, Schultz P, Pouyssegur J, Meijer L: **p42/p44 MAPKs are intracellular targets of the CDK inhibitor purvalanol.** *Oncogene* 2002, **21**:6413-6424.
 70. Dai Y, Dent P, Grant S: **Induction of apoptosis in human leukemia cells by the CDK1 inhibitor CGP74514A.** *Cell Cycle* 2002, **1**:143-152.
 71. Vermeulen K, Strnad M, Krystof V, Havlicek L, Van der Aa A, Lenjou M, Nijs G, Rodrigus I, Stockman B, van Onckelen H *et al.*: **Antiproliferative effect of plant cytokinin analogues with an inhibitory activity on cyclin-dependent kinases.** *Leukemia* 2002, **16**:299-305.
 72. Lane ME, Yu B, Rice A, Lipson KE, Liang C, Sun L, Tang C, McMahon G, Pestell RG, Wadler S: **A novel cdk2-selective inhibitor, SU9516, induces apoptosis in colon carcinoma cells.** *Cancer Res* 2001, **61**:6170-6177.
 73. Bain J, McLauchlan H, Elliott M, Cohen P: **The specificities of protein kinase inhibitors: an update.** *Biochem J* 2003, **371**:199-204.
 74. Dai Y, Yu C, Singh V, Tang L, Wang Z, McNistry R, Dent P, Grant S: **Pharmacological inhibitors of the mitogen-activated protein kinase (MAPK) kinase/MAPK cascade interact synergistically with UCN-01 to induce mitochondrial dysfunction and apoptosis in human leukemia cells.** *Cancer Res* 2001, **61**:5106-5115.
 75. Graves PR, Yu L, Schwarz JK, Gales J, Sausville EA, O'Connor PM, Piwnicka-Worms H: **The Chk1 protein kinase and the Cdc25C regulatory pathways are targets of the anticancer agent UCN-01.** *J Biol Chem* 2000, **275**:5600-5605.
 76. Monks A, Harris ED, Vaigro-Wolff A, Hose CD, Connelly JW, Sausville EA: **UCN-01 enhances the *in vitro* toxicity of clinical agents in human tumor cell lines.** *Invest New Drugs* 2000, **18**:95-107.
 77. Harvey S, Decker R, Dai Y, Schaefer G, Tang L, Kramer L, Dent P, Grant S: **Interactions between 2-fluoroadenine 9-beta-D-arabinofuranoside and the kinase inhibitor UCN-01 in human leukemia and lymphoma cells.** *Clin Cancer Res* 2001, **7**:320-330.
 78. Tang L, Boise LH, Dent P, Grant S: **Potential of 1-beta-D-arabinofuranosylcytosine-mediated mitochondrial damage and apoptosis in human leukemia cells (U937) overexpressing bcl-2 by the kinase inhibitor 7-hydroxystaurosporine (UCN-01).** *Biochem Pharmacol* 2000, **60**:1445-1456.
 79. Sato S, Fujita N, Tsuruo T: **Interference with PDK1-Akt survival signaling pathway by UCN-01 (7-hydroxystaurosporine).** *Oncogene* 2002, **21**:1727-1738.
 80. Fuse E, Hashimoto A, Sato N, Tanii H, Kuwabara T, Kobayashi S, Sugiyama Y: **Physiological modeling of altered pharmacokinetics of a novel anticancer drug, UCN-01 (7-hydroxystaurosporine), caused by slow dissociation of UCN-01 from human alpha1-acid glycoprotein.** *Pharm Res* 2000, **17**:553-564.
 81. Fry DW, Bedford DC, Harvey PH, Fritsch A, Keller PR, Wu Z, Dobrusin E, Leopold WR, Fattaey A, Garrett MD: **Cell cycle and biochemical effects of PD 0183812. A potent inhibitor of the cyclin D-dependent kinases CDK4 and CDK6.** *J Biol Chem* 2001, **276**:16617-16623.
 82. Soni R, O'Reilly T, Furet P, Muller L, Stephan C, Zumstein-Mecker S, Fretz H, Fabbro D, Chaudhuri B: **Selective *in vivo* and *in vitro* effects of a small molecule inhibitor of cyclin-dependent kinase 4.** *J Nat Cancer Inst* 2001, **93**:436-446.
 83. Sausville EA: **Complexities in the development of cyclin-dependent kinase inhibitor drugs.** *Trends Mol Med* 2002, **8**:S32-S37.
 84. Davies TG, Bentley J, Arris CE, Boyle FT, Curtin NJ, Endicott JA, Gibson AE, Golding BT, Griffin RJ, Hardcastle IR *et al.*: **Structure-based design of a potent purine-based cyclin-dependent kinase inhibitor.** *Nat Struct Biol* 2002, **9**:745-749.
- This study describes a potent purine-based CDK inhibitor discovered through a design based on the CDK2 crystal structure. This provides an excellent example of a novel CDK structure-based design of a potential antitumour agent.
85. Ikuta M, Kamata K, Fukasawa K, Honma T, Machida T, Hirai H, Suzuki-Takahashi I, Hayama T, Nishimura S: **Crystallographic approach to identification of cyclin-dependent kinase 4 (CDK4)-specific inhibitors by using CDK4 mimic CDK2 protein.** *J Biol Chem* 2001, **276**:27548-27554.
 86. Knockaert M, Meijer L: **Identifying *in vivo* targets of cyclin-dependent kinase inhibitors by affinity chromatography.** *Biochem Pharmacol* 2002, **64**:819-825.
- This study describes a new technique for identifying CDK inhibitor-binding molecules in cells. This approach could help to characterize the intracellular targets and selectivity of CDK inhibitors.